Supplementary Information

Recurrent activating *ACVR1* mutations in diffuse intrinsic pontine glioma

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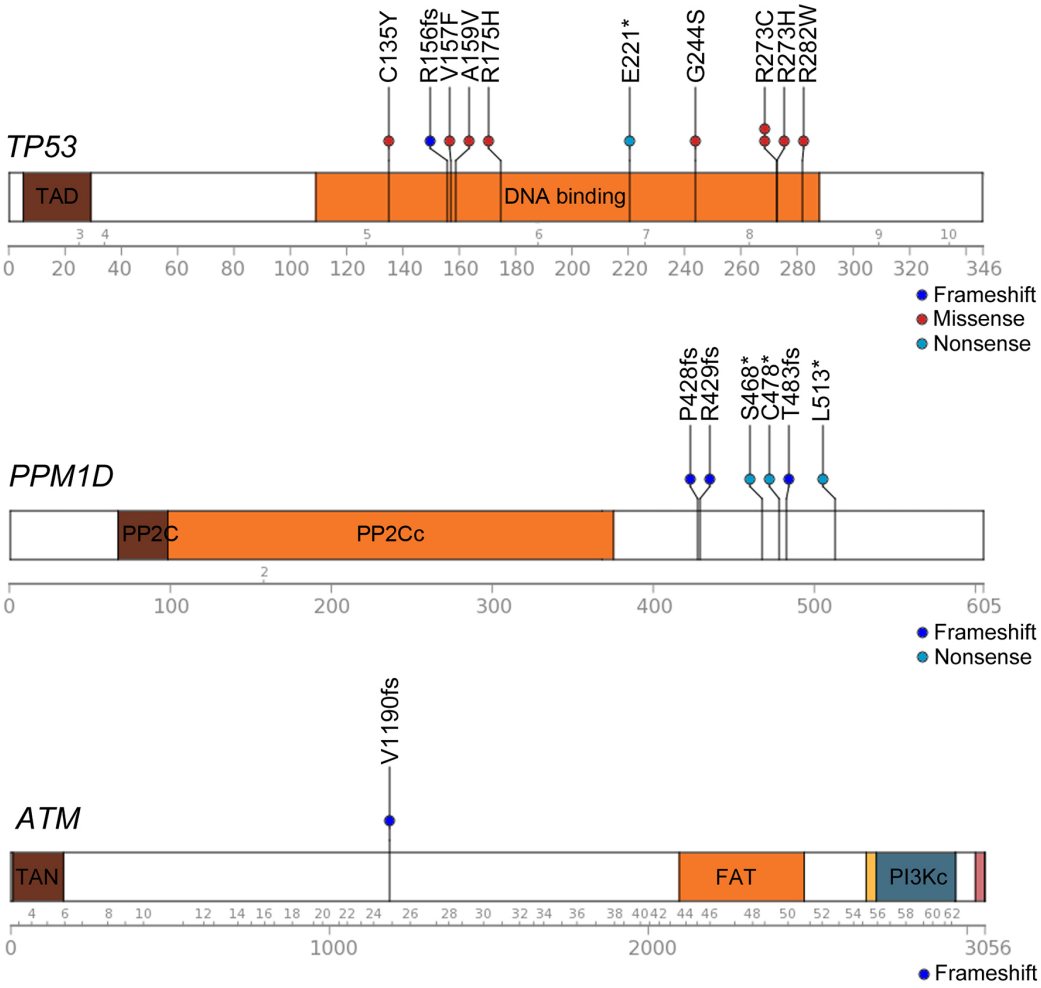
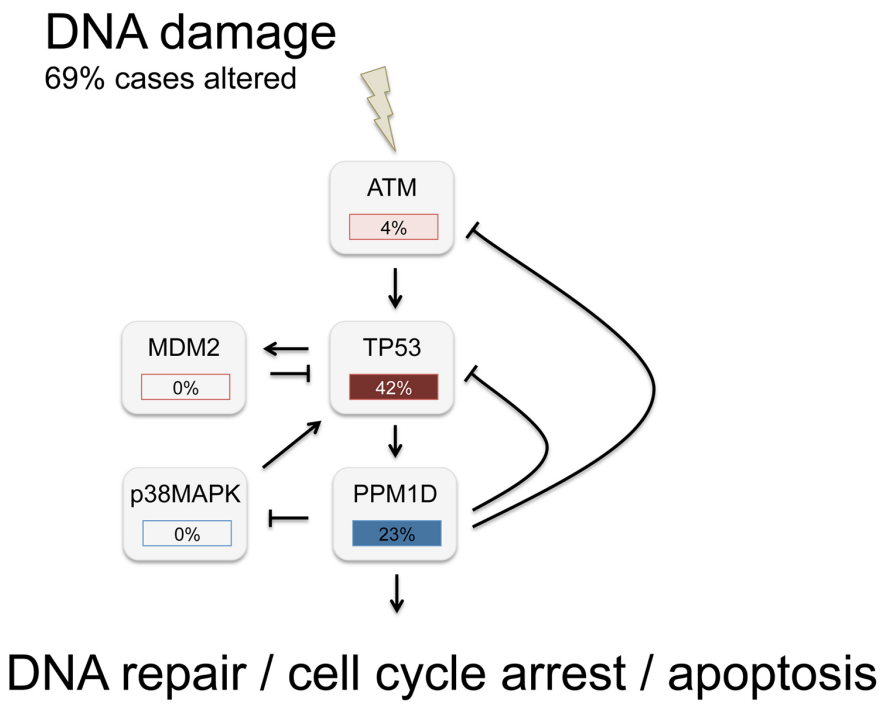
Supplementary Figures 1-7

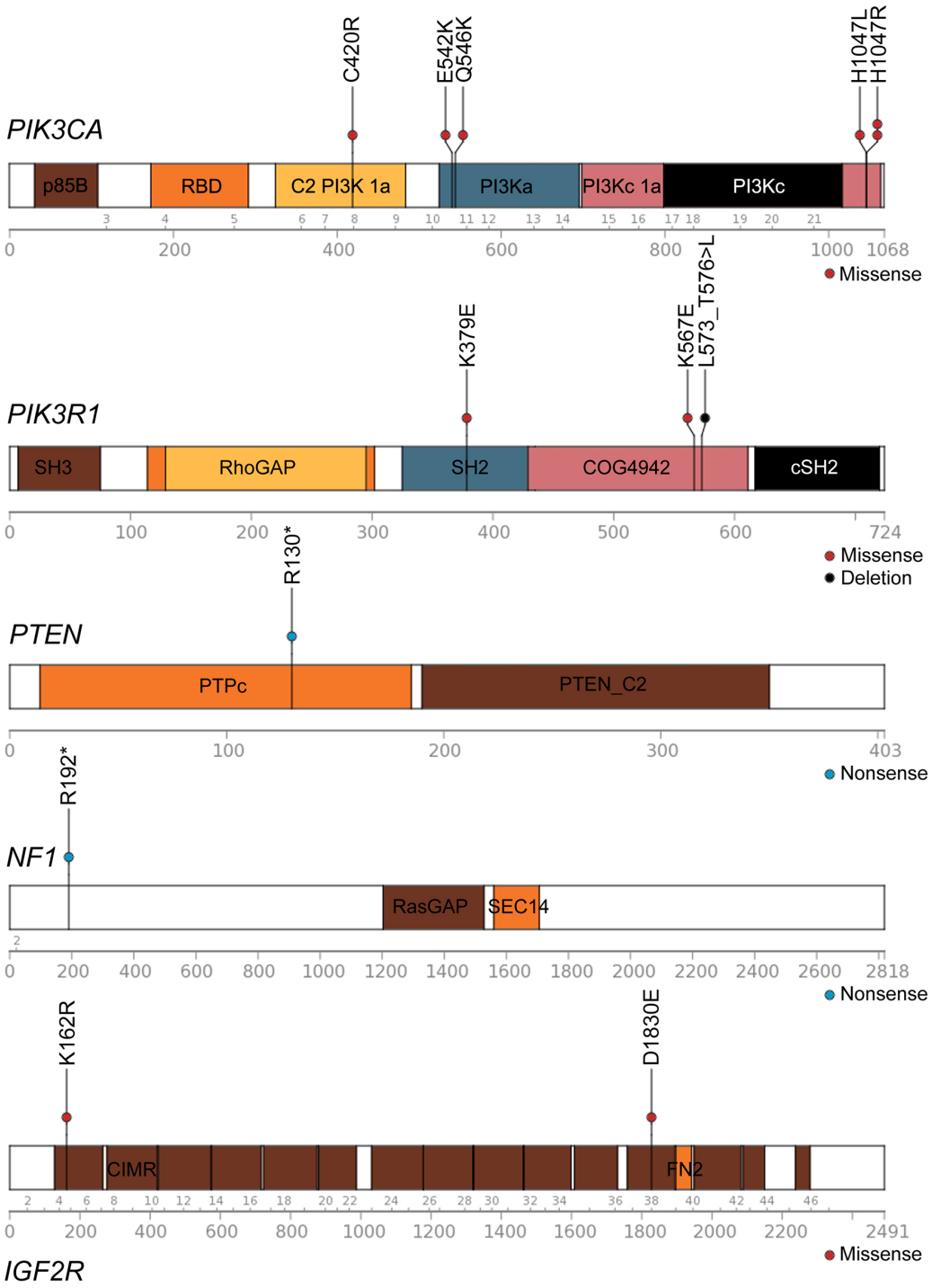
Supplementary Tables 2-4



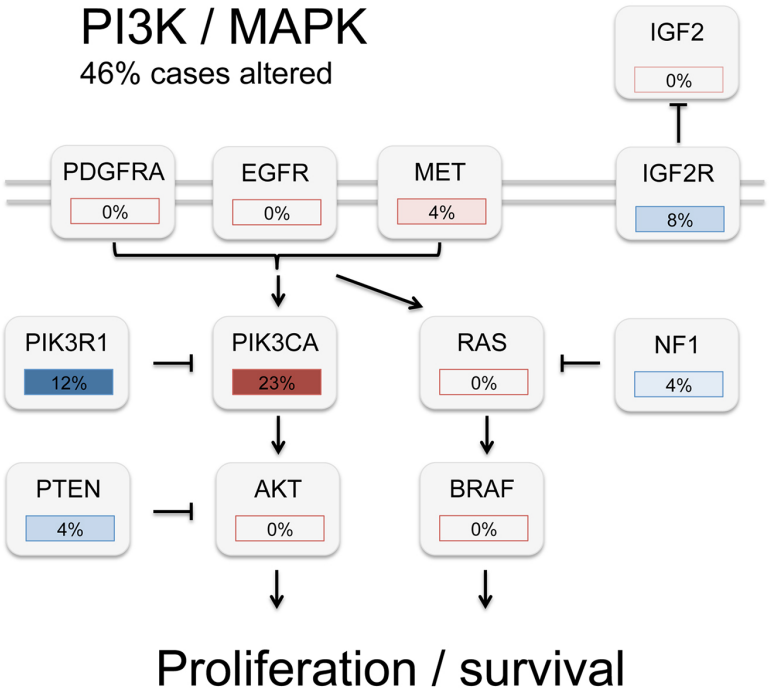
Supplementary Figure 1 – *Somatic mutations in TP53, PPM1D and ATM in DIPG.* Cartoon showing recurrent and non-overlapping missense and frameshift mutations in *TP53* (11/26, 42%), *PPM1D* (6/26, 23%) and *ATM* (1/26, 4%), overlaid with functional protein domains and exon boundaries. TAD: p53 transactivation motif; DNA binding: p53 DNA-binding domain; PP2C: protein phosphatase 2C domain; PP2Cc: Serine/threonine phosphatase, family 2C, catalytic domain. TAN: telomere length maintenance and DNA damage repair domain; FAT: FRAP, ATM and TRRAP associated domain; PI3Kc: phosphoinositide 3-kinase class I catalytic domain.



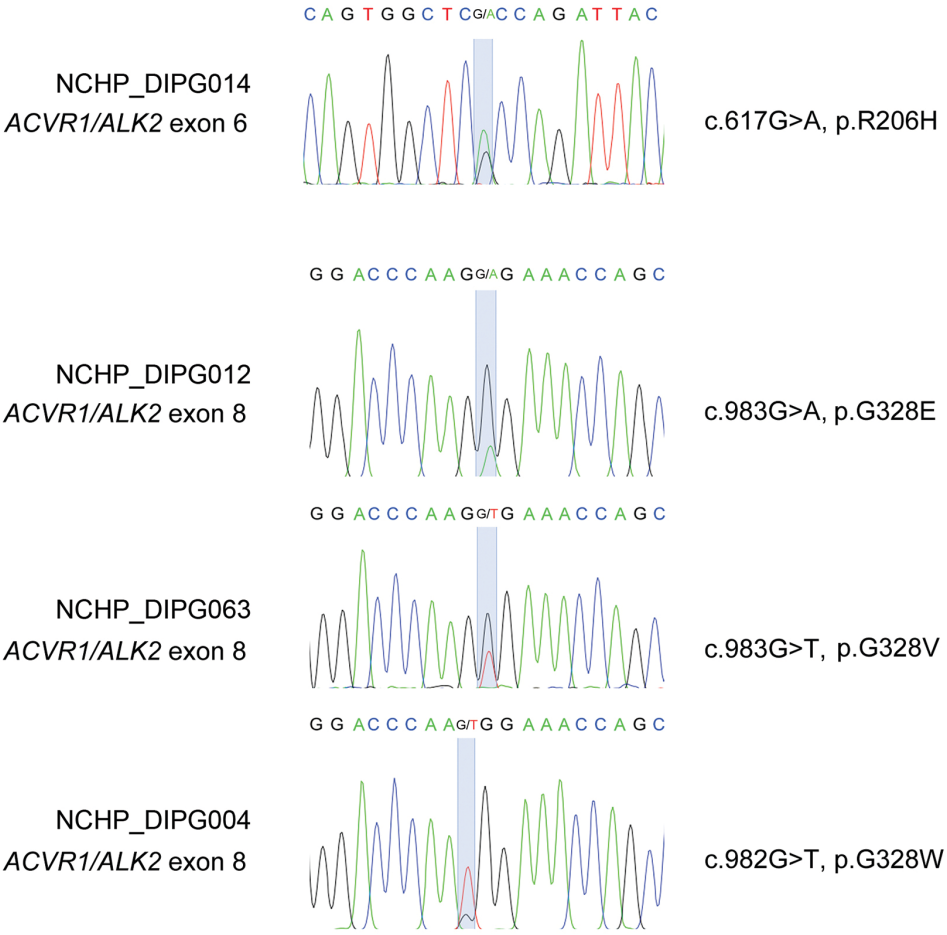
Supplementary Figure 2 – *Pathway-level recurrence of somatic alterations involved in DNA damage response.* Cartoon representing the frequency of distinct non-overlapping hits in intracellular components of ATM/p53-mediated DNA damage and stress response signalling. Bars are coloured according to frequency of alterations in the present cohort: red=gain of function, blue=loss of function. It total, 18/26 (69%) cases harboured alteration at some point in the pathway which would be predicted to be activating.



Supplementary Figure 3 – *Somatic mutations in genes involved in PI3K/MAPK signalling in DIPG.* Cartoon showing non-overlapping missense, nonsense truncating mutations, and deletions in *PIK3CA* (6/26,23%), PIK3R1 (3/26, 12%), PTEN (1/26, 4%) and *NF1* (1/26, 4%), overlaid with functional protein domains and exon boundaries. There were additional novel somatic mutations of unknown significance identified in *IGF2R*. Grey bars = deletions. p85B: p85 binding domain; RBD: Ras binding domain; C2 PI3K 1a: C2 domain present in class I alpha PI3 kinases; PI3Ka: PI3K class I accessory domain; PI3Kc: PI3K class I catalytic domain; SH: Src homology domain; RhoGAP: GTPase activator protein for Rho-like GTPases domain; COG4942: Membrane-bound metallopeptidase domain; PTPc: Protein tyrosine phosphatase, catalytic domain; PTEN_C2: PTEN C terminal 2 domain; RasGAP: Ras GTPase activating protein domain; SEC14: Sec14p-like lipid binding domain. CIMR: cation-independent mannose-6-phosphate receptor repeat; FN2: fibronectin type II domain.



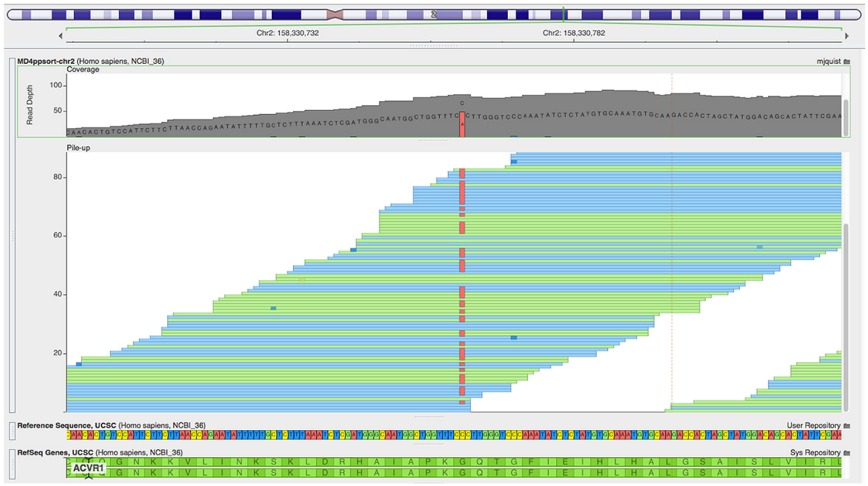
Supplementary Figure 4 – *Pathway-level recurrence of somatic alterations involved in RTK / PI3K / MAPK signalling.* Cartoon representing the frequency of distinct non-overlapping hits in intracellular components of PI3K/MAPK pathway signalling, as well as amplifications of receptor tyrosine kinases, in DIPG. Bars are coloured according to frequency of alterations in the present cohort: red=gain of function, blue=loss of function. IGF2R binds IGF2 ligand preventing signalling through IGF1R/PI3K, and is found to have a somatic missense K162R and D1830E mutations. It total, 12/26 (46%) cases harboured alteration at some point in the pathway which would be predicted to be activating.



Supplementary Figure 5 – *Sanger sequencing validation of ACVR1/ALK2 mutations in an extended cohort of DIPG.* Sequence traces of heterozygous mutations in the activin A type I receptor (*ACVR1*/*ALK2*) observed in a series of 50 DIPG, including (a) c.617G>A, R206H; (b) c.983G>A, p.G328E; (c) c.983G>T, p.G328V; (d) c.982G>T, p.G328W. All are reported to be constitutively activating of the BMP/TGF-β signalling pathway in models of fibrodysplasia ossificans progressiva.

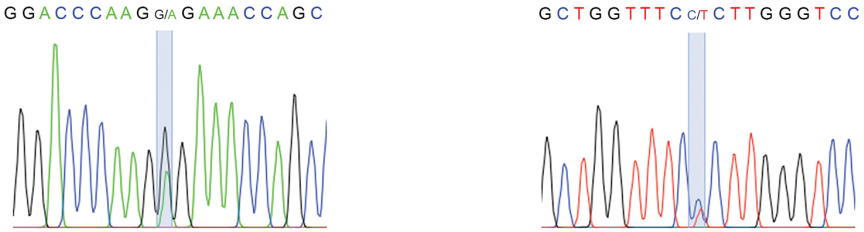
a

SU-DIPG-IV



b

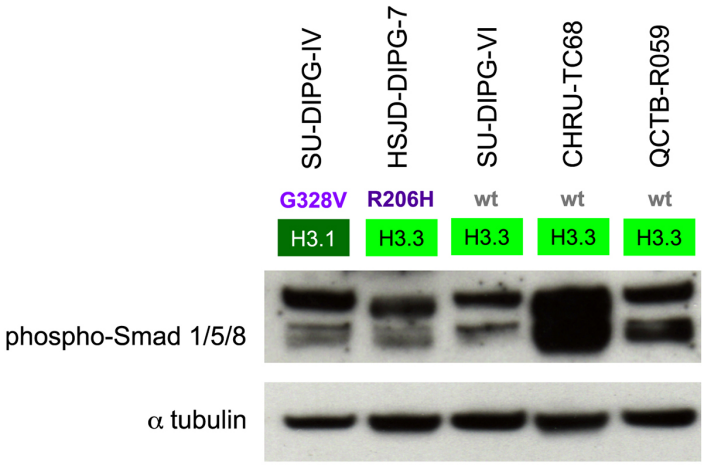
NCHP_DIPG011



Forward cDNA sequence

Reverse cDNA sequence

Supplementary Figure 6 – *Allele-specific expression of ACVR1/ALK2 mutation*. (a) Pile-up of sequence reads from RNAseq data of SU-DIPG-IV cells, showing expression of both the wild-type and mutant alleles at position chr2:158330762, with 49/83 reads harbouring the C>A (c.983G>T) mutation (red) corresponding to ACVR1/ALK2 p.G328V. (b) Sanger sequencing of an *ACVR1/ALK2* exon 8 RT-PCR product from DIPG patient sample NCHP_DIPG011, showing heterozygous expression of the mutant p.G328E allele (c.983G>A), forward and reverse.



Supplementary Figure 7 – *Basal levels of phospo-Smad 1/5/8 in DIPG cells*. Western blot analysis of phospo-Smad 1/5/8 (lower band) in SU-DIPG-IV (DIPG, *ACVR1/ALK2* G328V, *HIST1H3B* K27M), HSJD-DIPG007 (DIPG, *ACVR1/ALK2* R206H, *H3F3A* K27M), SU-DIPG-VI (DIPG, *ACVR1/ALK2* wt, *H3F3A* K27M), CHRU-TC68 (DIPG, *ACVR1/ALK2* wt, *H3F3A* K27M) and QCTB-R059 (thalamic paediatric GBM, *ACVR1/ALK2* wt, *H3F3A* K27M). α-tubulin is included as a loading control.

Study ID	Local ID	Hospital	Clinical diagnosis	Location	Age (yrs)	Sex	Histology	WHO	Source	Survival (months)	Outcome	Seq	Histone H3	ACVR1
NCHP_DIPG006	BAUK	Necker Childrens Hospital, Paris	DIPG	Pons	6.3	Male	GBM	4	Biopsy	8.3	Died	WGS	*H3F3A*	wt
NCHP_DIPG011	BOUC	Necker Childrens Hospital, Paris	DIPG	Pons	4.8	Female	AA	3	Biopsy	20.0	Died	WGS	*HIST1H3B*	G328E
NCHP_DIPG052	INAR	Necker Childrens Hospital, Paris	DIPG	Pons	4.6	Male	AA	3	Biopsy	10.2	Died	WGS	*HIST1H3B*	G328V
NCHP_DIPG061	MAHJ	Necker Childrens Hospital, Paris	DIPG	Pons	11.9	Female	LGA	2	Biopsy	5.0	Died	WGS	*H3F3A*	wt
NCHP_DIPG065	MJAY	Necker Childrens Hospital, Paris	DIPG	Pons	10.2	Male	GBM	4	Biopsy	16.8	Died	WGS	*H3F3A*	wt
NCHP_DIPG081	RUSL	Necker Childrens Hospital, Paris	DIPG	Pons	6.7	Male	GBM	4	Biopsy	16.8	Died	WGS	*H3F3A*	wt
NCHP_DIPG101	BAMN	Necker Childrens Hospital, Paris	DIPG	Pons	3.9	Female	GBM	4	Biopsy	13.1	Died	WGS	*H3F3A*	wt
NCHP_DIPG102	BENM	Necker Childrens Hospital, Paris	DIPG	Pons	10.3	Male	AOA	3	Biopsy	3.4	Died	WGS	*H3F3A*	wt
NCHP_DIPG103	DANA	Necker Childrens Hospital, Paris	DIPG	Pons	5.8	Female	GBM	4	Biopsy	17.5	Alive	WGS	*HIST1H3B*	wt
NCHP_DIPG104	DUJJ	Necker Childrens Hospital, Paris	DIPG	Pons	4.4	Male	LGA	2	Biopsy	9.1	Died	WGS	wt	wt
NCHP_DIPG105	GIBG	Necker Childrens Hospital, Paris	DIPG	Pons	6.6	Female	LGA	2	Biopsy	7.8	Died	WGS	*H3F3A*	wt
NCHP_DIPG106	HENJ	Necker Childrens Hospital, Paris	DIPG	Pons	12.1	Male	GBM	4	Biopsy	9.1	Died	WGS	wt	wt
NCHP_DIPG107	LACL	Necker Childrens Hospital, Paris	DIPG	Pons	8.8	Male	AA	3	Biopsy	8.4	Died	WGS	*H3F3A*	wt
NCHP_DIPG108	LEMN	Necker Childrens Hospital, Paris	DIPG	Pons	7.5	Male	AA	3	Biopsy	17.8	Alive	WGS	*HIST1H3B*	G328V
NCHP_DIPG109	MUCM	Necker Childrens Hospital, Paris	DIPG	Pons	6.2	Male	AOA	3	Biopsy	13.5	Died	WGS	*H3F3A*	wt
NCHP_DIPG110	PHIA	Necker Childrens Hospital, Paris	DIPG	Pons	5.7	Male	LGA	2	Biopsy	10.8	Died	WGS	wt	wt
NCHP_DIPG111	SCHL	Necker Childrens Hospital, Paris	DIPG	Pons	10.6	Female	AOA	3	Biopsy	8.6	Died	WGS	*H3F3A*	wt
NCHP_DIPG112	ZERR	Necker Childrens Hospital, Paris	DIPG	Pons	5.6	Female	LGA	2	Biopsy	13.3	Died	WGS	*H3F3A*	wt
NCHP_DIPG113	BLAG	Necker Childrens Hospital, Paris	DIPG	Pons	4.6	Male	AA	3	Biopsy	14.0	Died	WGS	*HIST1H3B*	G356D
NCHP_DIPG114	GONJ	Necker Childrens Hospital, Paris	DIPG	Pons	8.6	Male	LGA	2	Biopsy	20.3	Died	WGS	*HIST1H3B*	wt

HSJD_DIPG001	N06.48	Hospital Sant Joan de Déu, Barcelona	DIPG	Pons	6.0	Female	AA	3	Autopsy	11.0	Died	WES	*H3F3A*	wt
HSJD_DIPG002	N07.92	Hospital Sant Joan de Déu, Barcelona	DIPG	Pons	6.0	Female	AA	3	Autopsy	15.1	Died	WES	*HIST1H3B*	R258G
HSJD_DIPG003	N08.55	Hospital Sant Joan de Déu, Barcelona	DIPG	Pons	6.0	Male	GBM	4	Autopsy	6.6	Died	WES	*H3F3A*	wt
HSJD_DIPG004	N11.49	Hospital Sant Joan de Déu, Barcelona	DIPG	Pons	10.0	Female	LGA	2	Autopsy	35.3	Died	WES	*HIST1H3B*	G328E
HSJD_DIPG007	N12.32	Hospital Sant Joan de Déu, Barcelona	DIPG	Pons	9.9	Male	GBM	4	Biopsy	0.9	Died	WES	*H3F3A*	R206H
HSJD_DIPG008	HSJD-DIPG-8	Hospital Sant Joan de Déu, Barcelona	DIPG	Pons	6.5	Male	LGA	2	Autopsy	16.0	Died	WES	*H3F3A*	wt
NCHP_DIPG004	ADOM	Necker Childrens Hospital, Paris	DIPG	Pons	8.0	Female	LGA	3	Biopsy	26.0	Died	VAL	*HIST1H3B*	G328W
NCHP_DIPG008	BERG	Necker Childrens Hospital, Paris	DIPG	Pons	4.7	Male	AA	3	Biopsy	8.9	Died	VAL	*H3F3A*	wt
NCHP_DIPG012	BOUL	Necker Childrens Hospital, Paris	DIPG	Pons	4.5	Female	GBM	4	Biopsy	14.2	Died	VAL	*HIST1H3B*	G328V
NCHP_DIPG013	BOUM	Necker Childrens Hospital, Paris	DIPG	Pons	7.3	Female	AA	3	Biopsy	5.7	Died	VAL	*H3F3A*	wt
NCHP_DIPG014	BOUS	Necker Childrens Hospital, Paris	DIPG	Pons	6.2	Female	AA	3	Biopsy	1.2	Alive	VAL	wt	R206H
NCHP_DIPG025	CORC	Necker Childrens Hospital, Paris	DIPG	Pons	4.6	Male	AA	3	Biopsy	11.3	Died	VAL	*H3F3A*	wt
NCHP_DIPG026	CREA	Necker Childrens Hospital, Paris	DIPG	Pons	7.1	Male	LGA	2	Biopsy	12.3	Died	VAL	*H3F3A*	wt
NCHP_DIPG029	DECC	Necker Childrens Hospital, Paris	DIPG	Pons	3.4	Female	AA	3	Biopsy	14.1	Died	VAL	*H3F3A*	wt
NCHP_DIPG030	DECS	Necker Childrens Hospital, Paris	DIPG	Pons	13.6	Male	AA	3	Biopsy	9.3	Died	VAL	wt	wt
NCHP_DIPG032	DELT	Necker Childrens Hospital, Paris	DIPG	Pons	12.1	Male	AA	3	Biopsy	16.8	Died	VAL	*H3F3A*	wt
NCHP_DIPG043	GALC	Necker Childrens Hospital, Paris	DIPG	Pons	9.2	Male	AA	3	Biopsy	3.8	Died	VAL	wt	wt
NCHP_DIPG044	GALF	Necker Childrens Hospital, Paris	DIPG	Pons	9.4	Female	AA	3	Biopsy	8.3	Died	VAL	*H3F3A*	wt
NCHP_DIPG048	GVEE	Necker Childrens Hospital, Paris	DIPG	Pons	6.4	Female	AA	3	Biopsy	7.3	Died	VAL	*HIST1H3B*	wt
NCHP_DIPG050	HADZ	Necker Childrens Hospital, Paris	DIPG	Pons	6.5	Male	AA	3	Biopsy	7.2	Died	VAL	*H3F3A*	wt
NCHP_DIPG056	LEFL	Necker Childrens Hospital, Paris	DIPG	Pons	11.0	Male	AA	3	Biopsy	17.7	Died	VAL	*H3F3A*	wt

NCHP_DIPG062	MAKM	Necker Childrens Hospital, Paris	DIPG	Pons	10.8	Male	AA	3	Biopsy	12.3	Died	VAL	*H3F3A*	wt
NCHP_DIPG063	MAUM	Necker Childrens Hospital, Paris	DIPG	Pons	5.3	Female	GBM	4	Biopsy	14.9	Died	VAL	*HIST1H3B*	G328E
NCHP_DIPG069	NALF	Necker Childrens Hospital, Paris	DIPG	Pons	6.8	Female	GBM	4	Biopsy	6.0	Died	VAL	*H3F3A*	wt
NCHP_DIPG072	PAIC	Necker Childrens Hospital, Paris	DIPG	Pons	13.5	Female	GBM	4	Biopsy	10.9	Died	VAL	*H3F3A*	wt
NCHP_DIPG075	POIJ	Necker Childrens Hospital, Paris	DIPG	Pons	4.5	Female	GBM	4	Biopsy	20.4	Died	VAL	*HIST1H3B*	wt
NCHP_DIPG077	RAHR	Necker Childrens Hospital, Paris	DIPG	Pons	7.4	Female	AA	3	Biopsy	4.9	Died	VAL	*H3F3A*	wt
NCHP_DIPG079	RIER	Necker Childrens Hospital, Paris	DIPG	Pons	7.6	Male	GBM	4	Biopsy	14.1	Died	VAL	*H3F3A*	wt
NCHP_DIPG083	SANC	Necker Childrens Hospital, Paris	DIPG	Pons	11.3	Female	AA	3	Biopsy	14.2	Died	VAL	*H3F3A*	wt
NCHP_DIPG115	GREL	Necker Childrens Hospital, Paris	DIPG	Pons	1.7	Male	LGA	2	Biopsy	58.0	Died	VAL	*H3F3A*	wt
NCHP_DIPG116	HUPN	Necker Childrens Hospital, Paris	DIPG	Pons	4.4	Male	AOA	3	Biopsy	10.0	Died	VAL	wt	wt
NCHP_DIPG117	OWGC	Necker Childrens Hospital, Paris	DIPG	Pons	5.0	Male	AA	3	Biopsy	11.9	Died	VAL	*HIST1H3B*	wt

Supplementary Table 2 – *Description of samples used in this study.* Clinicopathological annotation of the 26 DIPG cases profiled by whole genome or exome sequencing in this study, as well as those used in the validation cohort. DIPG: diffuse intrinsic pontine glioma; GBM: glioblastoma multiforme; AA: anaplastic astrocytoma; AOA: anaplastic oligoastrocytoma; LGA: low grade astrocytoma. WGS: whole genome sequencing; WES: whole exome sequencing; VAL: validation.

Cell line ID	Originator	Clinical diagnosis	Location	Age (yrs)	Sex	Histology	WHO	Source	Survival (months)	Outcome	Histone H3	ACVR1
CHRU-TC68	Centre Hospitalier Régional Universitaire, Strasbourg	DIPG	Pons	9.8	Female	AA	3	Biopsy	7.0	Alive	*H3F3A*	wt
HSJD-DIPG007	Hospital Sant Joan de Déu, Barcelona	DIPG	Pons	9.9	Male	GBM	4	Biopsy	0.9	Died	*H3F3A*	R206H
QCTB-R059	Queensland Children's Medical Research Institute, Brisbane	GBM	Thalamus	10.4	Female	GBM	4	Surgery	0.9	Died	*H3F3A*	wt
SU-DIPG-IV	Stanford University, California	DIPG	Pons	3.0	Female	GBM	4	Autopsy	8.0	Died	*HIST1H3B*	G328V
SU-DIPG-VI	Stanford University, California	DIPG	Pons	7.0	Female	GBM	4	Autopsy	6.0	Died	*H3F3A*	wt

Supplementary Table 3 – *Details of primary cell cultures used.* Clinical and molecular data relating to the four DIPG and one H3F3A K27M mutant thalamic paediatric GBM cell cultures used for preclinical and mechanistic studies. DIPG: diffuse intrinsic pontine glioma; GBM: glioblastoma multiforme; AA: anaplastic astrocytoma.

Name	Forward	Reverse
H3F3A	GATTTTGGGTAGACGTAATCTTCA	TTTCCTGTTATCCATCTTTTTGTT
HIST1H3B	GGGCAGGAGCCTCTCTTAAT	ACCAAGTAGGCCTCACAAGC
ACVR1 exon 6	GATTGCTGCCCTTCATGTG	AAAAGCAGATTTTCCAAGTTCC
ACVR1 exon 7	TAATGATGGGCTGGCTGC	AAAACGGAGAGAGCAAAGGC
ACVR1 exon 8	GATGACATTTACTGTGTAGGTCGC	GATGCAACTCACCTAACCATTG
ACVR1 exon 9	TGGTTTAAAATCCTTCAGCAGC	TTTTAAAGGTAGCTGGATCAAGAG
ACVR1 exon 8 mRNA	TCAGGAAGTGGCTCTGGTCT	CAAGTCCAAAGGCCCAAATA

Supplementary Table 4 – *PCR primers used*. Sequences are given for PCR amplification of H3F3A, HIST1H3B and ACVR1 from genomic DNA for mutation detection, and ACVR1 from mRNA to determine allele-specific expression of the mutant.